Supporting Information

Binet and Maurelli 10.1073/pnas.0806768106

Isolation of *C. trachomatis* Cryptic Plasmid. Crude preparations of *C. trachomatis* L2 EBs collected from two 175-cm^2 infected flasks were washed 4 times with cold dH_2O, then subjected to 2 rounds of 30-min digestion with 10 units of RQ1 RNase-Free DNase (Promega). After centrifugation, the pellet was resuspended in 1 mg/mL of proteinase K in P1 buffer (QIAGEN) and incubated for 1 h at 65 °C. The cryptic plasmid was then extracted using the QIAGEN Plasmid Midi Kit procedure (QIAGEN) and resuspended in 100 μL of TE to a final concentration of 220 ng/μL. The methylation state of the plasmid DNA was determined by restriction analysis using enzymes sensitive to methylation by *E. coli* DAM methylase (BclI, MboI, DpnI), *E. coli* DCM methylase (MlsI, Bme1390I), or CG eukaryotic methylases (AclI, SmaI). The restriction analysis of *C. trachomatis* L2 cryptic plasmid, whose sequence is available in the GenBank database (accession no. X06707), suggested that the DNA was unmethylated (data not shown).

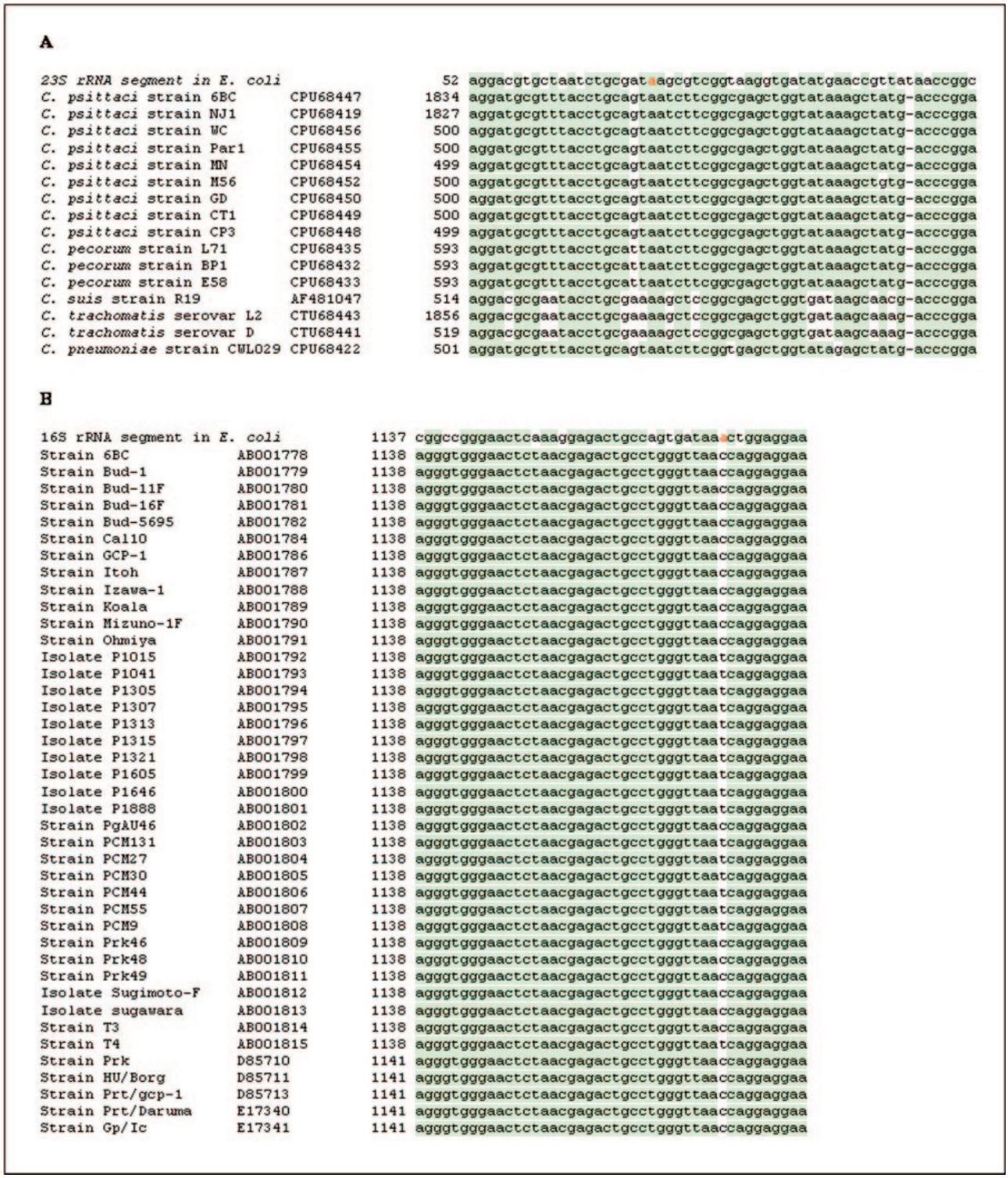
Detailed Experimental Procedures Leading to Purification of *C. psittaci* 6BC Recombinants. ***Before electroporation. Bacteria.*** Four 175-cm^2 flasks of confluent L2 mouse fibroblast cells infected at a multiplicity of infection of 1 with *C. psittaci* 6BC were lysed at about 46 h postinfection (hrs p.i.) and submitted to multiple centrifugation through RenoCal-76 (Bracco Diagnostics) density gradients as described by Caldwell *et al.* (1) with slight modifications. Infected cells were removed with glass beads, pooled, and ruptured by sonication and centrifuged at 15,000 × g for 30 min at 4 °C. The pellet was resuspended in 8 mL SPG (250 mM sucrose, 10 mM sodium phosphate, 5 mM L-glutamic acid) and layered over 5.4 mL of a 30% (vol/vol) RenoCal-76 solution, and then centrifuged at 24,000 rpm for 40 min at 4 °C in a SW40 Ti rotor. The pellet was resuspended in 1 mL SPG and layered over discontinuous RenoCal-76 gradients (3 mL 40%, 5 mL 44%; 4 mL 54% RenoCal-76, vol/vol). This gradient was centrifuged at 20,000 rpm for 40 min at 4 °C in a SW40 Ti rotor. The EB band located at the 44/54% Renocal interface was collected, diluted in SPG, and then centrifuged at 24,000 rpm for 30 min at 4 °C. Highly purified preparations of *C. psittaci* 6BC EBs were subsequently resuspended into 500 μL SPG, split in 25 μl aliquots and frozen at −80 °C until analysis. Chlamydial stocks prepared this way typically contain more than 10^{10} PFU/mL.

DNA. Plasmids were extracted from *E. coli* grown in 200 mL LB following the Maxi Kit procedure (Qiagen), resuspended in 600 μL TE buffer, and further purified by 3 phenol and chloroform extractions before ethanol-precipitation. DNA was resuspended in dH_2O to a final concentration of about 1 μg/μL.

Transformation. One aliquot of bacteria was thawed on ice. Ten microliters of EBs was gently mixed in 1 mL ice-cold dH_2O using a pipetman, centrifuged at 4 °C at 10,000 rpm for 10 min, and resuspended in ice-cold dH_2O to a final concentration of $\approx 10^9$ PFU/mL. Ten microliters EBs ($\approx 10^7$ PFUs) in dH_2O was mixed with DNA in water to a final volume of 60 μL, transferred to cold 0.1-cm electroporation cuvettes, and electroporated at 1.6 kV, 600 Ω, 25 μF, using a Gene Pulser (Bio-Rad). Two successive pulses were applied before addition of 450 μL 1× DMEM. The transformation mixture was transferred to a microcentrifuge tube before infection of tissue culture cells.

Selection of recombinants. Confluent monolayers of L2 mouse fibroblast cells in 60 mm dishes were washed twice with prewarmed (37 °C) 1× DMEM, then covered with 550 μL prewarmed 1X DMEM before bacterial infection. Three dishes (1–3) were infected with 100 μL of bacteria diluted to 10^{-4} in cold 1× DMEM; 3 dishes (4–6) were infected with 100 μL, 200 μL, or the rest of the transformation mixture, respectively. All 6 dishes were rocked at 37 °C in 5% CO_2 on a rocker platform set at 2.5 (Bellco Glass Inc.). After 2 h of infection, dishes 4–6 were washed once with prewarmed 1× DMEM, then incubated with 3 mL of recovery medium [1× DMEM, 10% FBS, 1× NEM nonessential amino acids (NEAA; Sigma-Aldrich), and 0.2 μg/mL cycloheximide] at 37 °C in 5% CO_2 for 15–16 additional hours. After the 2 h infection for dishes 1–3, or a total of 16–17 h for dishes 4–6, the inoculum was replaced with 5 mL of an agarose overlay containing 0.75% sterile Seakem GTG agarose (FMC Bioproducts) prewarmed to 55 °C, mixed with an equal volume of prewarmed (37 °C) 2× DMEM containing 20% FBS, 0.4 μg/mL cycloheximide, 2× NEAA and antibiotics (40 μg/mL of gentamicin for dishes 1–3; 6 mg/mL KSM, 600 μg/mL Spc, and 40 μg/mL of gentamicin for dishes 4–6). At day 7, a second 5 mL agarose overlay containing the same components as the first was added. Generally, at 10 days p.i. the cells in dishes 1–3 were stained with 0.5% neutral red for 3 h at 37 °C in 5% CO_2 to visualize the chlamydial plaques and determine the bacterial titers, including the pretransformation titer corresponding to unelectroporated bacteria, and the posttransformation (survival) titers. At the same time, overlays were gently removed from dishes 4–6 before plaque scoring, purification, and expansion with selection at 2 h p.i. Alternatively, selection of transformants was done at 2 h p.i. after a postelectroporation antibiotic-free passage for 1 development cycle.

1. Caldwell HD, Kromhout J, Schachter J (1981) Purification and partial characterization of the major outer membrane protein of *Chlamydia trachomatis*. *Infect Immun* 31:1161–1176.



A

23S rRNA segment in E. coli		52	aggacgtgctaatctgcgat**a**agcgtcggtaaggtgatatgaaccgttataaccggc
C. psittaci strain 6BC	CPU68447	1834	aggatgcgtttacctgcagtaatcttcggcgagctggtataaagctatg-acccgga
C. psittaci strain NJ1	CPU68419	1827	aggatgcgtttacctgcagtaatcttcggcgagctggtataaagctatg-acccgga
C. psittaci strain WC	CPU68456	500	aggatgcgtttacctgcagtaatcttcggcgagctggtataaagctatg-acccgga
C. psittaci strain Par1	CPU68455	500	aggatgcgtttacctgcagtaatcttcggcgagctggtataaagctatg-acccgga
C. psittaci strain MN	CPU68454	499	aggatgcgtttacctgcagtaatcttcggcgagctggtataaagctatg-acccgga
C. psittaci strain M56	CPU68452	500	aggatgcgtttacctgcagtaatcttcggcgagctggtataaagctgtg-acccgga
C. psittaci strain GD	CPU68450	500	aggatgcgtttacctgcagtaatcttcggcgagctggtataaagctatg-acccgga
C. psittaci strain CT1	CPU68449	500	aggatgcgtttacctgcagtaatcttcggcgagctggtataaagctatg-acccgga
C. psittaci strain CP3	CPU68448	499	aggatgcgtttacctgcagtaatcttcggcgagctggtataaagctatg-acccgga
C. pecorum strain L71	CPU68435	593	aggatgcgtttacctgcattaatcttcggcgagctggtataaagctatg-acccgga
C. pecorum strain BP1	CPU68432	593	aggatgcgtttacctgcattaatcttcggcgagctggtataaagctatg-acccgga
C. pecorum strain E58	CPU68433	593	aggatgcgtttacctgcattaatcttcggcgagctggtataaagctatg-acccgga
C. suis strain R19	AF481047	514	aggacgcgaatacctgcgaaaagctccggcgagctggtgataagcaacg-acccgga
C. trachomatis serovar L2	CTU68443	1856	aggacgcgaatacctgcgaaaagctccggcgagctggtgataagcaaag-acccgga
C. trachomatis serovar D	CTU68441	519	aggacgcgaatacctgcgaaaagctccggcgagctggtgataagcaaag-acccgga
C. pneumoniae strain CWL029	CPU68422	501	aggatgcgtttacctgcagtaatcttcggtgagctggtatagagctatg-acccgga

B

16S rRNA segment in E. coli		1137	cggccgggaactcaaaggagactgccagtgataa**c**tggaggaa
Strain 6BC	AB001778	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Bud-1	AB001779	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Bud-11F	AB001780	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Bud-16F	AB001781	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Bud-5695	AB001782	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Cal10	AB001784	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain GCP-1	AB001786	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Itoh	AB001787	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Izawa-1	AB001788	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Koala	AB001789	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Mizuno-1F	AB001790	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Ohmiya	AB001791	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Isolate P1015	AB001792	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Isolate P1041	AB001793	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Isolate P1305	AB001794	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Isolate P1307	AB001795	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Isolate P1313	AB001796	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Isolate P1315	AB001797	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Isolate P1321	AB001798	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Isolate P1605	AB001799	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Isolate P1646	AB001800	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Isolate P1888	AB001801	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Strain PgAU46	AB001802	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain PCM131	AB001803	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Strain PCM27	AB001804	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Strain PCM30	AB001805	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Strain PCM44	AB001806	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Strain PCM55	AB001807	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Strain PCM9	AB001808	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Strain Prk46	AB001809	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Prk48	AB001810	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Prk49	AB001811	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Isolate Sugimoto-F	AB001812	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Isolate sugawara	AB001813	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain T3	AB001814	1138	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain T4	AB001815	1138	agggtgggaactctaacgagactgcctgggttaatcaggaggaa
Strain Prk	D85710	1141	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain HU/Borg	D85711	1141	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Prt/gcp-1	D85713	1141	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Prt/Daruma	E17340	1141	agggtgggaactctaacgagactgcctgggttaaccaggaggaa
Strain Gp/Ic	E17341	1141	agggtgggaactctaacgagactgcctgggttaaccaggaggaa

Fig. S1. Conservation of *C. psittaci* 6BC 23S rRNA A_{72} base and 16S rRNA C_{1071} base. (*A*) Sequence alignment of the chlamydial 23S rRNA region with *E. coli* 23S rRNA nucleotide 52–108. Each chlamydial strain is followed by the respective GenBank accession number used in the alignment and the corresponding starting position. *E. coli* A_{72} is highlighted in red. (*B*) Sequence alignment of the 16S rRNA region of 42 *C. psittaci* strains *with E. coli* 16S rRNA nucleotide 1137–1180. Each *C. psittaci* strain is followed by the respective GenBank accession number used in the alignment and the corresponding starting position. *E. coli* A_{1071} is highlighted in red

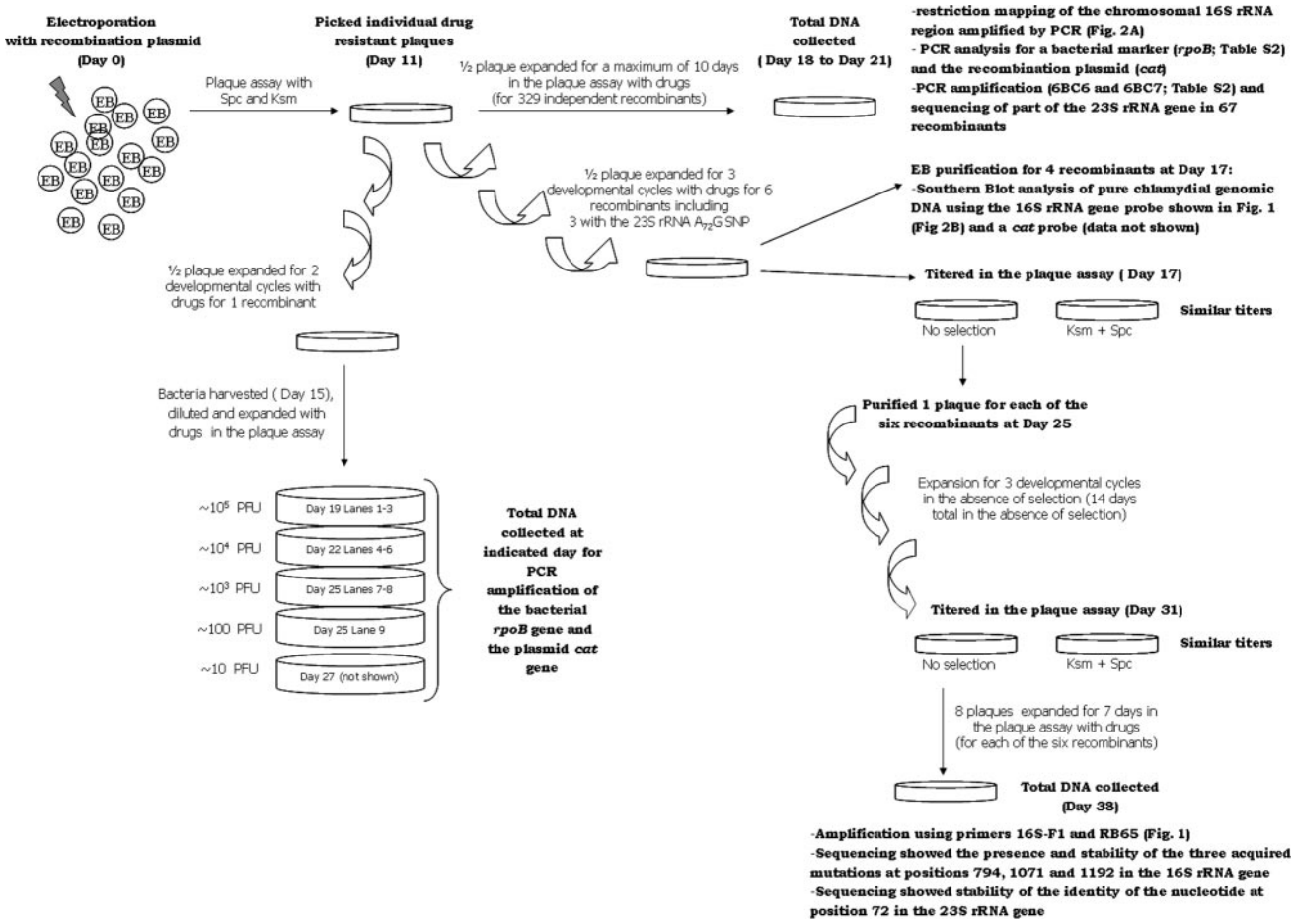


Fig. S2. Schematic representation and timeline of the serial expansion of *C. psittaci* 6BC recombinants leading to the various analyses reported in this study.

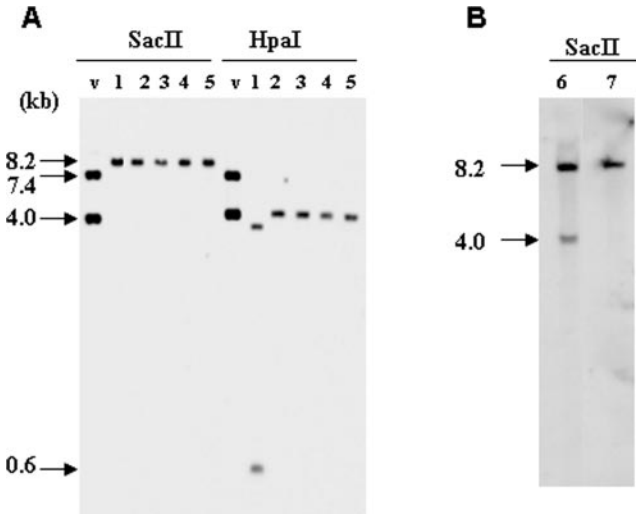


Fig. S3. Southern hybridization analyses of *C. psittaci* 6BC recombinants. (*A*) Southern hybridization of *C. psittaci* 6BC pure genomic DNA using the 16S rRNA and the *cat* probes. The membrane shown in Fig. 2*C* (main text) which was hybridized with the 16S probe, was hybridized with a probe for the *cat* gene carried by the transformation vector. The recombination plasmid pRAK426 (lanes v) shows the presence of the 7.4-kb SacII *cat* fragment, the 4.0-kb SacII 16S fragment, the 7.2-kb HpaI *cat* fragment, and the 4.3-kb HpaI 16S fragment in the recombination plasmid pRAK426 (lanes v). SacII-digested genomic DNA from the parent *C. psittaci* 6BC strain (lanes 1), and the 4 pRAK426 derived recombinants (lanes 2–5) revealed a single 8.2-kb (16S) band, whereas HpaI digested genomic DNA showed 2 (16S) bands for the parent strain and a single band (16S) for the 4 recombinants. (*B*) Southern hybridization of total DNA from cells infected with 2 recombinants prepared at 18 days posttransformation with the 16S probe shown in Fig. 1. Although these 2 DNA lysates showed the presence of the recombination plasmid by PCR (data not shown), only the sample in lane 6 revealed the vector 4.0 kb SacII band in addition to the 8.2 kb SacII 16S chromosomal fragment. This finding confirms that Southern hybridization was not as sensitive as PCR in detecting episomal carriage of the recombination plasmid.

Table S1. Bacterial strains and plasmids used in this study

Strains and plasmids	Description	Source or reference
Strain		
C. trachomatis L2	Biovar lymphogranuloma venereum L2/434/Bu	H. Caldwell
C. psittaci 6BC	Strain 6BC	T. Hatch
BC_{RB}	Clonal *C. psittaci* strain 6BC	(1)
BC0E1	Spontaneous Spc^R variant of *C. psittaci* 6BC with a $C_{1192}T$ mutation in the 16S rRNA gene; Spc^R	(2)
BCKS1	Spontaneous Ksm^R variant of BC0E1 with $A_{794}G$ and $C_{1192}T$ mutations in the 16S rRNA gene; Ksm^R, Spc^R	This work
E. coli		
DH5α	F^- *ϕ80Δ(lacZY-argF)*U169 *deoR recA1 endA1 phoA hsdR17 supE44* λ^- *thi-1 gyrA96 relA1* Δ*(lacZ)M15*	(3)
GM272	F^- *fhuA2 or fhuA31 lacY1 or lacZ4 tsx-1 or tsx-78 glnV44(AS) galK2(Oc)* λ^- *dcm-6 dam-3 mtlA2*	Coli genetic stock collection
JC12	Hfr *fhuA1 lacY1 or lacZ4 tsx-1 glnV44 (AS) gal-6* λ*- rfbC1 purF1 mtlA2 metB1 xyl-7*	Coli genetic stock collection
EC100	F^- *araD139* Δ*(ara, leu)7697* φ*80 DlacZ*Δ*M15* Δ*lacX74 recA1 pir+(DHFR) galK galU* λ*- endA1 mcrA rpsL nupG* Δ*(mrr- hsdRMS - mcrBC)*	Epicentre
Plasmid		
pGEMT	ColE1 (pMB1) Ori, Amp^R	Promega
pBC SK (+)	ColE1 (pMB1) Ori, Cm^R	Stratagene
pPCR-script Cam SK (+)	ColE1 (pMB1) Ori, Cm^R	Stratagene
pRAK392*	Part of *ftsK* amplified with Platinum *taq* high-fidelity DNA polymerase (Invitrogen) using FtsKF1 and FtsKR6 and ligated in pGEMT	
pRAK216*	Region upstream to 16S rRNA gene amplified with Platinum *taq* high-fidelity DNA polymerase using 6BC4 and 6BC5 and ligated into pGEMT	
pRAK183*	16S rRNA gene amplified with Platinum *taq* high-fidelity DNA polymerase using 16SF1 and 16SR1and cloned in pGEMT (2)	
pRAK270*	*C. psittaci* BCKS1 16S rRNA gene amplified with Platinum *taq* high-fidelity DNA polymerase using 16SF1 and 16SR1 and cloned in pGEMT	
pRAK419*	Site-directed mutagenesis of pRAK270 using mut7-F and mut7-R	
pRAK220*	Partial 23S rRNA gene and full 5S rRNA gene amplified with Accupol DNA polymerase (Gene Choice) using 6BC8 and 6BC9 cut with BstEII, ligated with partial 23S rRNA gene amplified using 6BC6 and 6BC7 cut with BstEII and cloned in pPCR-script Cam SK (+)	
pRAK215*	Partial 23S rRNA gene and full 5S rRNA gene amplified with Accupol DNA polymerase using 6BC8 and 6BC9 and cloned in pPCR-script Cam SK (+)	
pRAK217†	SphI fragment from pRAK183 ligated into SphI site of pRAK216, screened for correct orientation	
pRAK390†	NheI XmaI fragment from pRAK270 ligated into NheI XmaI fragment of pRAK217	
pRAK221†	BstEII PshAI fragment from pRAK215 ligated into BstEII PshAI fragment of pRAK220	
pRAK404†	NotI SwaI fragment from pRAK292 ligated into NotI SwaI fragment of pRAK390	
pRAK407†	XmaI SalI fragment from pRAK404 ligated into XmaI SalI fragment of pRAK221	
pRAK426†	NheI XmaI fragment from pRAK419 ligated into NheI XmaI fragment of pRAK407	
pRAK410†	ClaI BmgBI fragment from pRAK407 ligated into ClaI SmaI fragment of pBCSK (+)	
pRAK412†	XhoI NheI fragment from pRAK407 ligated into XhoI NheI fragment of pRAK410.	
pRAK429†	SphI NgoMIV fragment from pRAK424 ligated into SphI NgoMIV fragment of pRAK223	
pRAK418†	BstEII BamHI fragment of pRAK412, filled in with Klenow *exo* minus and self ligated	
pRAK424†	NheI XmaI fragment from pRAK419 ligated into NheI XmaI fragment of pRAK418	
pRAK417†	BmgBI FspI fragment of pRAK412 self ligated	
pRAK423†	NheI XmaI fragment from pRAK419 ligated into NheI XmaI fragment of pRAK417	
pRAK427†	BmgBI FspI fragment from pRAK424 self ligated	
pRAK411†	SpeI BglII fragment from pRAK407 ligated into SpeI BamHI fragment of pBCSK (+)	
pRAK428†	pRAK418 cut with NheI BsrGI ligated into pRAK411 cut with NheI BsrGI	

*Plasmids generated by PCR cloning using *C. psittaci* 6BC wild-type as template unless specified.
†Plasmids generated by subcloning (enzymes from New England Biolabs).

1. Binet R, Maurelli AT (2007) Frequency of development and associated physiological cost of azithromycin resistance in Chlamydia psittaci 6BC and C. trachomatis L2. *Antimicrob Agents Chemother* 51:4267–4275.
2. Binet R, Maurelli AT (2005) Frequency of spontaneous mutations that confer antibiotic resistance in Chlamydia spp. *Antimicrob Agents Chemother* 49:2865–2873.
3. Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* 166:557–580.

Table S2. Primers used for polymerase chain reaction amplification, site-directed mutagenesis, and sequencing

Primer target and designation	Position*	Sequence (5′ → 3′)
C. psittaci 6BC rRNA region†		
ftsK-R9	−360	GCATTTGTATTAAGATTTGACGAGG
ftsK-R6	17	CATGTCTCCATTCCCCAT
6BC4 (NotI added-)	2094	ATAGCGGCCGC-TAATACTTGGTTATATCAAGAATGG
ftsK-F1	2560; C	GCGCTGGTTTCTTAGACGTACCTG
16S-F1	3040	AGAATTTGATCTTGGTTCAGATTG
6BC5	3326; C	CCATGCTGACTTGACGTCA
16S1	3673	GCATCTAATACTATCTTTCTAGAGGG
RT2	4065	TTTCCGCAAGGACAGATACACAG
RT1	4181; C	ACCCTAAGTGTTGGCAACTAACG
6BC6	4219	AAGGCGAGGATGACGTCAAGT
16S8	4364; C	GTCGAGTTGCAGACTACAATCC
16S-R1	4623; C	CCTAGTCAAACCGTCCTAAGACAG
RB65	4996; C	TGTCGCCTTATACGCCTATG
6BC8	6448	AGCTGTTGATGGTGACCGTAC
6BC7	6472; C	TTAGGTACGGTCACCATCAACAG
6BC9	8107; C	CACCAGAAATCAGTCAGACAA
Mut7-F	4193	CGAGACTGCCTGGGTTAATCAGGAGGAAGGCGAGG
Mut7-R	4216; C	CCTCGCCTTCCTGATTAACCCAGGCAGTCTCG
C. psittaci 6BC *fol* region‡		
rpoD2§	1051592; C	ACATATGCCACTTGGTGGATCCGTCA
recA2§	1018875	AATGAGGGGATTTCTTCAGCAGGATG
Fol18 (EcoRI added-)	1614	CGGAATTC-ATGATGTTGAAGCAAACCACAGGG
Fol20 (XbaI added-)	2118; C	AGGCTCTAGA-GTTAAGTCTCTTTCTCATCTAAAACAGT
C. psittaci 6BC *rpoB*¶		
rpoB61-F	667	TTCTCAAGTACACCGTTCTCCAG
rpoBRT9	1217; C	GAGCATTTGCCAAGGTCGTAG
*aadA***		
aadA-F	2241	TGCAAGTAGCGTATGCGCTCAC
aadA-R	3411; C	TTGTGTAGGGCTTATTATGCAC
aadA-F RT	2711	CGAGATTCTCCGCGCTGTA
aadA-R RT	2777; C	TGGATAACGCCACGGAATG
cat††		
cat-F	2138	TCACTGGATATACCACCGTTGA
catM-R	2507; C	CCGTAACACGCCACATCTTG

*Position in GenBank entry for the first base of the primer. C, complementary strand.
†Position in pRAK426 insert sequence (GenBank accession no. EU871431) is indicated (Fig. 1).
‡The *C. psittaci* 6BC 3926 bp *rpoD* to *recA* genomic region containing *folA* was sequenced after PCR amplification by using Platinum *taq* high-fidelity DNA polymerase (Invitrogen) with primer rpoD2 and primer recA2 and cloning into pGEMT-easy (Promega). The sequence was deposited in GenBank under accession no. EU871432.
§Designed based on the DNA sequence of the respective homologous gene present in the genome sequences of the *C. muridarum* (reference for primer position), *C. pneumoniae* strain AR39, and *C. trachomatis* serovar D (GenBank accession nos. AE002160, AE002161, and AE001273, respectively).
¶Designed using GenBank accession no. AY826976 for *C. psittaci* 6BC *rpoB* sequence.
**Designed using GenBank accession no. M69063 for pAM34 sequence.
††Designed using GenBank accession no. U46018 for pCR-script Cam sequence.

Table S3. Relationship between recombination frequency and physical state of the recombination donor plasmid DNA

	Recombination donor plasmid DNA	*C. psittaci* recombinants*	
Physical state	Amount, μg	Highest frequency	Highest number
Methylated† and linear‡	50	2.4×10^{-6}	2
	20	4.3×10^{-6}	2
	10	1.7×10^{-6}	2
	5	$> 3.1 \times 10^{-7}$	0
Unmethylated§ and linear‡	10	1.0×10^{-6}	2
	5	5.2×10^{-7}	1
Methylated† and circular	20	0.5×10^{-6}	2
	10	1.1×10^{-6}	4
	5	0.5×10^{-6}	2
Unmethylated§ and circular	20	2.9×10^{-6}	14
	10	2.7×10^{-6}	14
	5	0.4×10^{-6}	3

*The optimal results out of a minimum of 3 independent experiments are presented.
†Prepared from *E. coli* strains DH5α, JC12, or EC100 (Table S1).
‡Double-stranded NotI-digested pRAK407 or pRAK426.
§Prepared from *E. coli* strain GM272 (Table S1).